

# Genotoxicity of *N*-Acetylarylamines in the Salmonella/Hepatocyte System

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Coincubation of isolated, intact rat hepatocytes with *Salmonella typhimurium* tester strain TA 98 (Salmonella/hepatocyte system) has been employed to determine both bacterial mutagenicity and DNA damage in the hepatocytes following treatment with 2-acetylaminofluorene (AAF) and other AAF derivatives. *In vivo* pretreatment of rats with either 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) or 3-methylcholanthrene (MC) markedly increased both DNA damage and bacterial mutation frequency upon incubation of AAF or 2-aminofluorene (AF), in this system. The increase in damage to the hepatocyte-DNA was more pronounced after AAF treatment than by that for AF, while the increase in bacterial mutation frequency was greater after AF treatment. Hepatocytes treated with paraoxon prior to exposure to *N*-hydroxy-2-acetylaminofluorene (*N*-OH-AAF) or *N*-acetoxy-2-acetylaminofluorene (*N*-OAc-AAF) partially inhibited the DNA damage caused by these agents, as well as inhibiting the bacterial mutagenicity of *N*-OAc-AAF (50% inhibition) and *N*-OH-AAF (80% inhibition). These data indicate that metabolic processes other than those involved in activating AAF and its derivatives into a bacterial mutagen(s) may contribute to the genotoxic effects of these compounds. Thus, the Salmonella/hepatocyte system may provide a useful model in which to study the relative role of the various metabolic processes associated with the carcinogenic effects of *N*-acetylarylamines.

## Introduction

Carcinogenic *N*-acetylarylamines, such as 2-acetylaminofluorene (AAF), are extensively metabolized in animals that are both resistant and susceptible to the carcinogenic effects of these compounds (1). In the organism, metabolic processing of *N*-acetylarylamines involves both detoxification and activation (toxification), and there is convincing evidence that the ratio between these two processes may, to a large extent, determine the susceptibility to carcinogenesis from these compounds (2, 3).

Recent studies on the mechanism of AAF-induced hepatocarcinogenesis have clearly established that the oncogenic process proceeds via initiation and promotion phases (4). AAF is a complete rat liver carcinogen that can provoke initiation as well as maintain the promotional aspects of the carcinogenic process in this species. It thus seems reason-

able to ask which of the metabolic pathways involved in AAF metabolism can convert this compound into derivatives that are responsible for these two phases of AAF-induced carcinogenesis. This is of particular interest with respect to AAF as the obligatory *N*-hydroxylation step is followed by several activation pathways (Fig. 1).

Previous work from our laboratory indicated that deacetylation, either by membrane bound deacetylase(s) or by cytosolic *N*-*O*-acyltransferase, was the most important step in the mutagenic activation of *N*-hydroxy-2-acetylaminofluorene (*N*-OH-AAF) in the Salmonella system (5). One drawback to the use of subcellular fractions such as S-9 and microsomes in these metabolic activation experiments is the lack of metabolic detoxification processes that can occur in the intact organism. Therefore the balance between metabolic activation and detoxification that ultimately determines the toxic effects *in vivo* is not reflected. In order to correct this imbalance we have recently devised a system employing intact rat hepatocytes (or any other cell type) in combination with Salmonella tester strains (Salmonella/hepatocyte system) to study the genotoxic effects of known or suspected carcinogens (6). In this system

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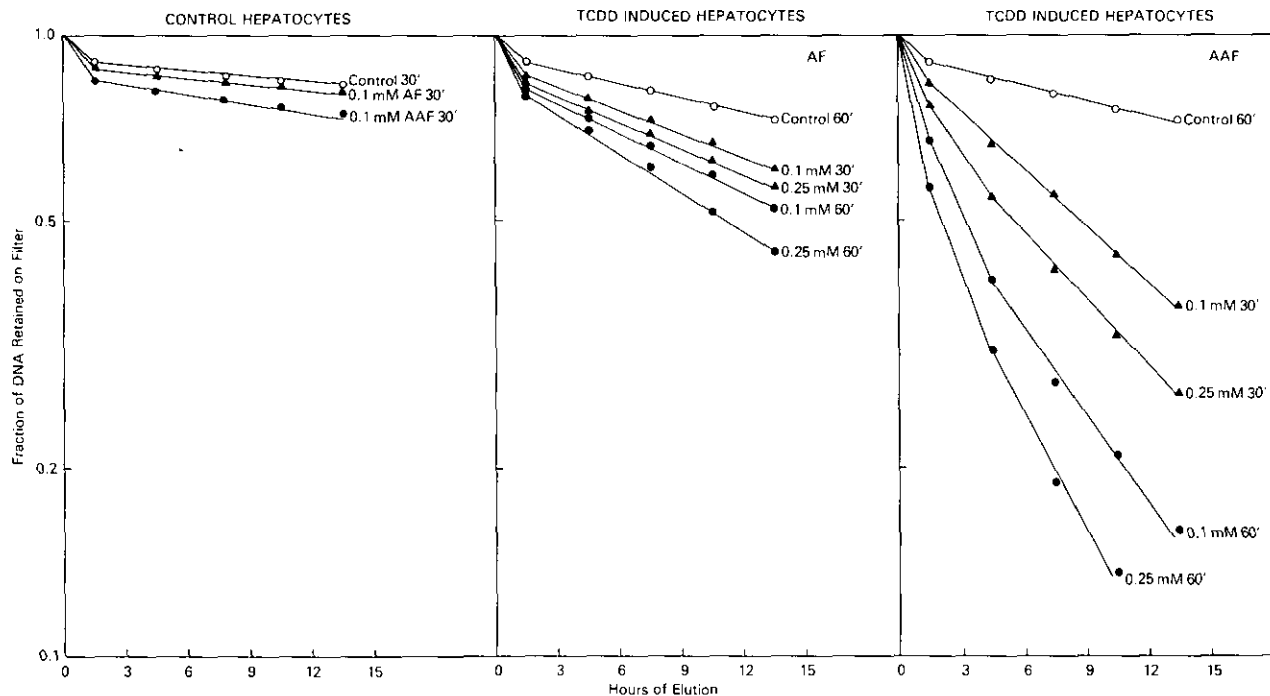


FIGURE 2. Effects of TCDD induction on DNA damage by AF and AAF in the *Salmonella*/hepatocyte system. Rats were treated with 50  $\mu\text{g/kg}$  IP of TCDD 48 hr prior to isolation of hepatocytes.

the increase in mutagenicity is much greater for AF than it is for AAF (Fig. 3). It is possible that a single metabolite of AF and/or AAF is responsible for bacterial mutation, while DNA damage in the hepatocyte, as measured by alkaline elution, may result from the additive effect of more than one reactive metabolite.

Previous work has shown that *N*-hydroxy-2-aminofluorene (N-OH-AF) is the mutagenic species derived from N-OH-AAF in the *Salmonella* system (5). N-OH-AF is a direct frameshift mutagen in tester strain TA 98 as first shown by Ames et al. (11, 12). Therefore it is reasonable to assume that N-OH-AF is the bacterial mutagen derived from both AF and AAF in the *Salmonella*/hepatocyte system. However, other reactive metabolites derived from AAF such as the sulfate ester of N-OH-AAF or N-acetoxy-AF (see Fig. 1) may contribute to the DNA damage either by direct interaction with DNA, or by causing general cytotoxic effects. As a result, cellular DNA may become sensitive to the alkaline condition employed.

Paraoxon (diethyl-*p*-nitrophenyl phosphate) is an effective inhibitor of the microsomal deacetylase responsible for activation of N-OH-AAF to a frameshift mutagen (i.e., N-OH-AF) (12). However it does not inhibit the mutagenic activation of N-OH-AAF by the cytosolic *N*-O-acyltransferase (14). The use of this inhibitor thus provides an important tool in

evaluating the relative importance of deacetylation and *N*-O-acyltransfer in the metabolic activation of N-OH-AAF. Figures 4 and 5 show that *in vitro* pretreatment of isolated hepatocytes with paraoxon is capable of partially inhibiting the DNA damage caused by both N-OH-AAF and N-OAc-AAF. This indicates that metabolic processes in addition to paraoxon-sensitive deacetylation contribute to N-OH-AAF and/or N-OAc-AAF-induced DNA damage in normal rat hepatocytes. The mutation frequency caused by both N-OH-AAF and N-OAc-AAF in strain TA 98 is also inhibited by paraoxon (Figs. 6 and 7). The inhibition of mutagenicity is more pronounced for N-OH-AAF (80-90% inhibition) than for N-OAc-AAF (<50%). However, the inhibition of N-OH-AAF and N-OAc-AAF mutagenicity observed at lower paraoxon concentrations (<10<sup>-6</sup> M) disappears at the highest concentration of paraoxon used (10<sup>-4</sup> M). The reason for this effect of paraoxon at the highest concentration is presently unclear. It is possible that the inhibitory effects of paraoxon are not limited to the deacetylase at the higher concentrations employed here.

Characterization of metabolic processes that are critically involved in the activation and detoxification of chemical carcinogens must be considered before any attempt to define the carcinogenic process is undertaken. Use of intact cells from *in vivo* target organ(s) for a particular chemical carcinogen pro-

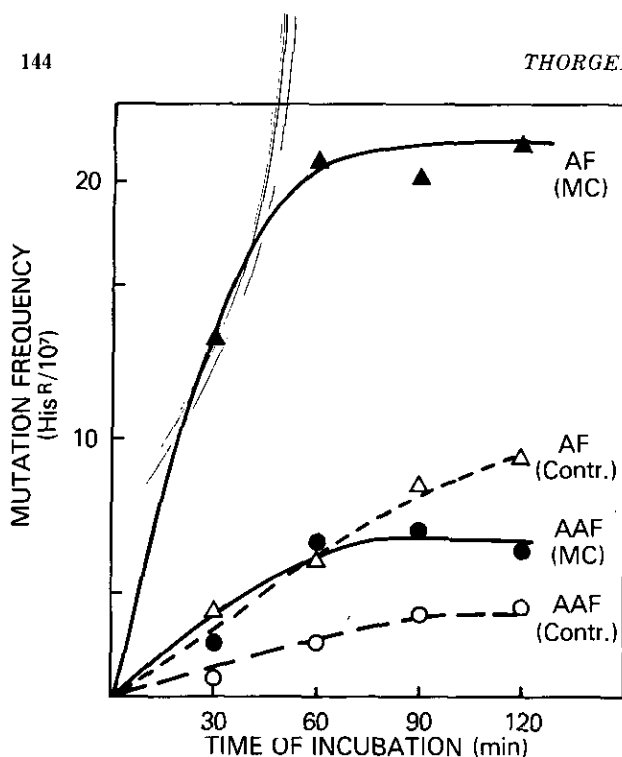


FIGURE 3. Effect of MC induction on mutagenicity of AF and AAF in the *Salmonella*/hepatocyte system. Rats were treated with 80 mg/kg IP of MC 48 hr prior to isolation of hepatocytes. Concentration of both AAF and AF was 250  $\mu$ M; tester strain TA 98 was used.

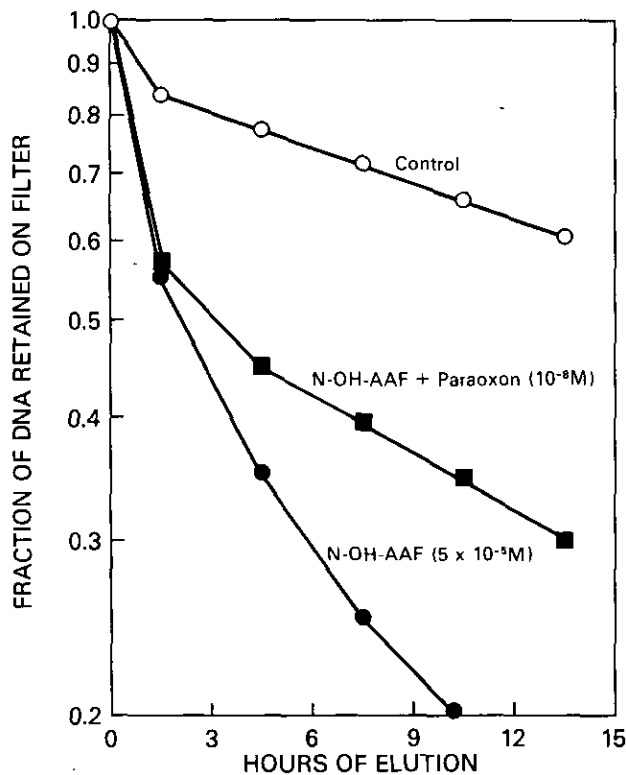


FIGURE 4. Inhibition of N-OH-AAF induced DNA damage by paraoxon. Paraoxon was added to the hepatocytes 15 min prior to the 30 min incubation with N-OH-AAF.

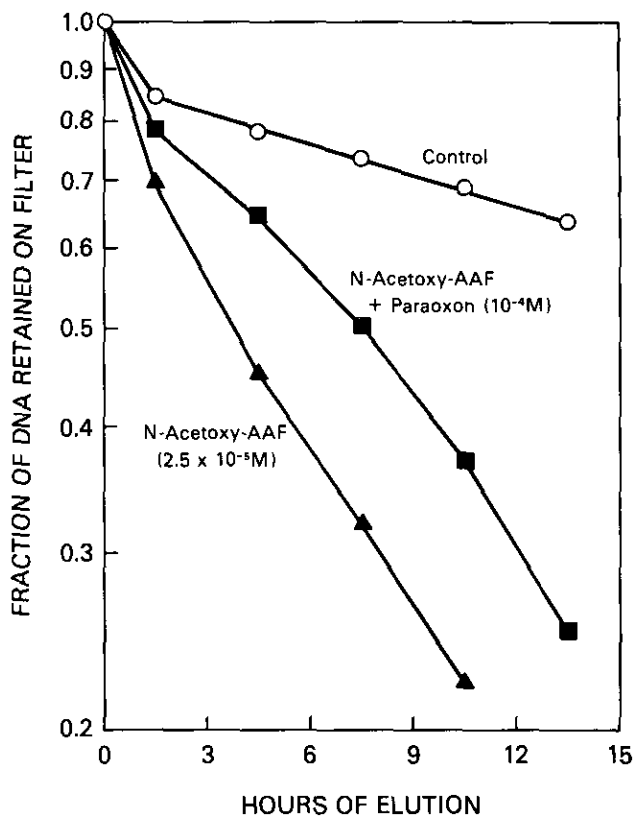


FIGURE 5. Inhibition of N-OAc-AAF induced DNA damage by paraoxon. Experimental conditions as described in legend for Figure 4.

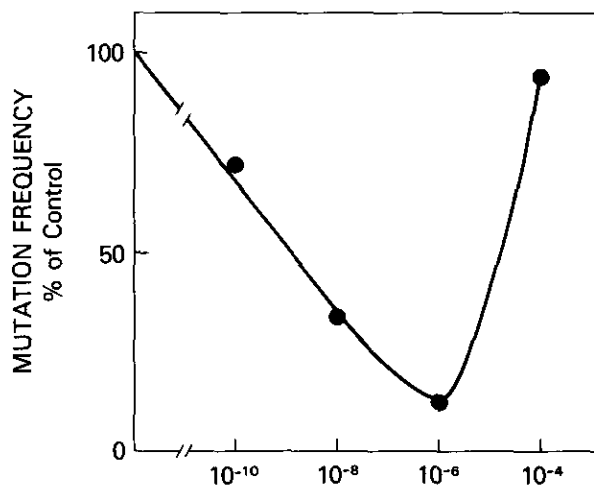


FIGURE 6. Effect of paraoxon on N-OH-AAF mutagenicity in the *Salmonella*/hepatocyte system. Paraoxon was added to the hepatocytes 15 min prior to the addition of bacteria and N-OH-AAF (100  $\mu$ M). The complete system was incubated for 30 min.

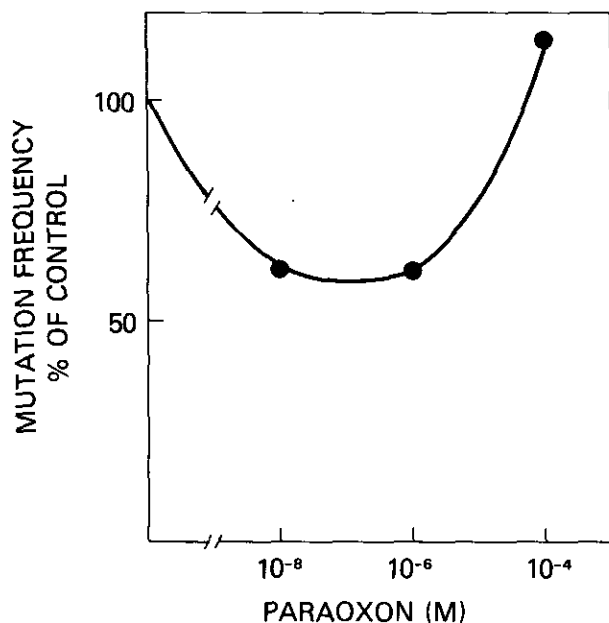


FIGURE 7. Effect of paraoxon on N-OAc-AAF mutagenicity in the *Salmonella*/hepatocyte system. Experimental conditions as described in legend to Figure 6, except that the concentration of N-OAc-AAF was 50  $\mu$ M.

vides a way to study in a comprehensive fashion the metabolic processes involved in carcinogenesis.

Therefore the *Salmonella*/hepatocyte system may provide an important new model for studying the relative roles of different metabolic pathways in the initiation and promotion of hepatocarcinogenesis by N-acetylarylamines.

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